USSN: 09/616,283; Art Unit: 1645
Attorney Docket No. VRXB-P01-001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

GOODNOW

Serial No: 09/616,283

Filed: July 14, 2000

For: SYSTEM FOR DETECTING BACTERIA IN BLOOD, BLOOD PRODUCTS, AND FLUIDS OF TISSUES

Art Unit: 1645

Attorney Docket No. VRXB-P01-001

Examiner: J. Hines

Assistant Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration Under 35 U.S.C. §1.132

Sir:

I, Jeffrey A. Hall, Ph.D., of Franklin, MA, hereby declare as follows:

1. I am the Director of Assay Development at Verax Biomedical, Inc., the assignee of the present application. I have been conducting research in immunoassay development in the field of testing blood, blood products, and tissue for 13 years. Accordingly, my curriculum vitae is attached as Appendix A.

2. I have read the above-identified application, the pending claims, the Office Action mailed on February 11, 2003, and the Advisory Action mailed on June 19, 2003.

3. I understand that the Examiner has stated that the invention as described and claimed in the above-identified application is obvious in view of the teachings of Chan (EP 461,462), McLaughlin (U.S. Patent 4,683,196), Tadler et al. (*J. Clin. Lab. Anal.* 3: 21-25 (1989)), and Chang et al. (U.S. Patent 5,200,323).

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4. The Examiner states that McLaughlin teaches antibodies which specifically bind to gram negative bacteria in order to determine their presence and/or absence while Tadler et al., teach well known binding agents that bind lipotechoic acid of gram-positive bacteria in assays. See Office Action dated February 11, 2003. Accordingly, the Examiner states that it would have been "prima facie obvious to modify the simultaneous multiple analyte detection immunoassay taught by Chan by incorporating a set of binding agents as taught by McLaughlin and Tadler et al."

5. I have reviewed the disclosures of both Tadler et al. and McLaughlin. For the reasons set forth below and the accompanying experimental data presented, I believe that the antibodies in these documents fail to demonstrate broad pan-generic cross-reactivity and detection at a level of sensitivity to be effective in detecting clinically relevant amounts of bacteria in a blood or blood products.

6. Verax Biomedical, Inc., has developed pan-generic antibodies immunoreactive with the Gram-negative antigen lipopolysaccharide (LPS) and pan-generic antibodies immunoreactive with the Gram-positive antigen lipoteichoic acid (LTA).

7. McLaughlin discloses mouse and rabbit antibodies against gram-negative bacteria such as *Neisseria, Chlamydia, and Salmonella*. However, the McLaughlin antibodies fails to demonstrate the broad range of cross-reactivity demonstrated by the Verax antibodies. In particular, the Verax antibodies show pan-generic activity against a range of bacteria that have been identified as contanimants in blood and blood products in three major national transfusion reaction studies including the BaCon Study in the United States, the

Hemovigilance study in France, and the SHOT study in the United Kingdom. These contaminants include both pathogenic as well as non-pathogenic bacterial species such as *Yersinia entercolitica* and *Proteous miribilis*, along with other common soil-borne bugs. The McLaughlin antibodies appear to be effective mainly against the pathogenic gram negative bacterial species.

8. In view of the patents and literature citations above, we have conducted a side-by-side comparison of the Verax antibodies to antibodies that are commercially available from HyCult Biotech, Virostat, QED, and Biogenesis, Inc., that are marketed as binding the LPS on Gram-negative bacterium and as binding to the LTA on Gram-positive bacterium.

9. For example, McLaughlin discloses antibodies that are capable of binding the LPS on Gram-negative bacteria. McLaughlin states that these antibodies bind to the Lipid A core of the Gram-negative bacteria. We obtained commercially available mouse antibodies that are marketed as being anti-LPS core or anti-endotoxin [Appendix B] and conducted a side-by-side comparison to the Verax antibodies. These results are presented below:

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PAN-GENERA REACTIVITIES (S:N RATIO) OF VARIOUS BINDING AGENTS TOWARDS GRAM NEGATIVE BACTERIA

TEST BACTERIA

Vendor	Antigen	*Enterobacter cloacae*	*Enterobacter aerogenes*	*Acinetobacter baumanii*	*Klebsiella oxytoca*	*Klebsiella pneumoniae*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella enteritidis*	*Yersinia enterocolitica*	*Proteus mirabilis*	*Serratia marcescens*
HyCult Biotech	LPS	1	1	1.1	1.2	1.1	1.2	1	1	1	1.3	1.3
Virostat	LPS	1	1	1	1	1	1	1	1	1	1	1.1
QED	LPS	1	1	1	1	1	1	NT	NT	NT	NT	NT
VERAX PGD BA-1	LPS	12.1	11.1	12.5	12.8	12.3	9.8	8.7	11.2	11.4	10.6	12.3
VERAX PGD BA-2	LPS	11.2	16.5	14.2	9.5	7.8	13.5	9.2	8.5	12.1	19.5	22.7

* "SAMPLE-TO-NOISE" RATIO = ANTIGEN-SPECIFIC SIGNAL/BACK-GROUND SIGNAL.
** S:N RATIO IS A COMMON EIA DATA NORMALIZATION TECHNIQUE TO SIMULTANEOUSLY COMPARE REACTIVITIES OF MULTIPLE BINDING AGENTS. A S:N RATIO >2 IS REQUIRED TO CONSTRUCT A MEANINGFUL ASSAY.

Antibodies immunoreactive with the Gram-negative bacterial antigen LPS were tested against 11 strains of Gram-negative bacterium routinely found in contaminated blood. The allegedly pan-generic antibodies from HyCult Biotech, Virostat, and QED, were not found to be truly pan-generic with respect to their ability to detect Gram-negative bacterium.

As can be seen from the results above, a signal: noise ratio greater than 2 is required for constructing a meaningful assay. The Verax antibodies showed greater effectiveness and could be detected in an immunoassay as compared to the commercially available antibodies.

10. The sensitivity of the pan-generic gram-negative Verax antibodies is set forth below:

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VERAX PLATELET PGD ASSAY: ANALYTICAL SENSITIVITY

	GRAM NEGATIVE RAPID TEST SIGNAL (G/DENS)										
CFU/ml	*E. coli*	*Ps. aeruginosa*	*E. aerogenes*	*K. oxytoca*	*E. cloacae*	*A. baumanii*	*K. pneumoniae*	*S. enteritidis*	*P. mirabilis*	*S. marcescens*	CFU/ml
1.0 E5	4.51	2.16	8.13	8.88	1.01	7.71	5.30	1.14	1.38	0.83	1.0 E5
5.0 E4	4.10	1.45	7.70	7.08	0.77	7.51	4.40	0.70	0.98	0.71	5.0 E4
1.0 E4	2.76	0.79	6.88	3.31	0.82	4.79	2.34	0.50	0.58	0.69	1.0 E4
5.0 E3	1.34	0.87	4.81	1.46	0.73	2.98	1.21	0.48	0.24	0.16	5.0 E3
1.0 E3	0.99	0.32	4.10	0.25	0.75	1.48	0.57	0.04	0.09	0.01	1.0 E3

11. Further, Tadler et al disclose antibodies cross-reactive with the lipoteichoic acid (LTA) of the Gram-positive bacteria. Tadler et al. state that their antibodies are capable of binding a large number of Gram-positive bacteria. However, their experimental data is limited to binding and detection of 5 species of gram-positive bacteria. In contrast, Verax antibodies are capable of pangeneric binding and detection of at least 11 Gram-positive bacterial species.

We obtained commercially available mouse antibodies that are marketed as being anti-LTA [Appendix C] and conducted a side-by-side comparison to the Verax gram positive antibodies. These results are presented below:

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PAN-GENERA REACTIVITIES (S:N RATIO) OF VARIOUS BINDING AGENTS TOWARDS GRAM POSITIVE BACTERIA

		TEST BACTERIA								
Vendor	Antigen	Staph epidermidis	Staphylococcus aureus	Staph lugdenensis	Bacillus subtilis	Group B Streptococcus	Group G Streptococcus	Enterococcus faecalis	Corynebacterium sps	Clostridium perfringes
HyCult Biotech	G+ LTA	28.1	1.2	1.4	1.3	1.5	3.8	1.9	3.8	1.7
Biogenesis Inc.	G+ LTA	9.2	16.3	1.1	2.9	1.5	6.7	1.3	6.7	2.2
VERAX PGD BA-3	G+ LTA	62.6	7.6	12.5	20.3	5.6	14.8	5.8	14.8	4.7
VERAX PGD BA-4	G+ LTA	77.9	30.1	71.3	10.2	6.6	10.8	21.4	10.7	2.8

* "SAMPLE-TO-NOISE" RATIO = ANTIGEN-SPECIFIC SIGNAL/BACK-GROUND SIGNAL
** S:N RATIO IS A COMMON EIA DATA NORMALIZATION TECHNIQUE TO SIMULTANEOUSLY COMPARE REACTIVITIES OF MULTIPLE BINDING AGENTS. A S:N RATIO >2 IS REQUIRED TO CONSTRUCT A MEANINGFUL ASSAY.

The antibodies from HyCult Biotech and Biogenesis, Inc. were not truly pan-generic with respect to the detection of Gram-positive bacterium. In contrast, the Verax antibodies detected 11 species of bacterium of Gram-positive bacterium routinely found in contaminated blood. As can be seen from the results above, to be effective in detecting clinically relevant amounts of bacteria a signal: noise ratio greater than 2 is required for constructing a meaningful assay. The Verax antibodies showed greater effectiveness and could be detected in an immunoassay as compared to the commercially available antibodies.

12. Tadler et al. also demonstrate the sensitivity of their immunoassays. Figure 2 shows that only 2 bacterial species, i.e., *Streptococcus mutans* and *Staphylococcus epidermidis*, are

detected at $5x10^5$ CFU/50µl (i.e., $1x10^7$ CFU/ml); and further that *Staphylococcus aureus* is detected at $5x10^6$ CFU/50µl (i.e., $1x10^8$ CFU/ml), a level that is not clinically relevant. Additionally, the Tadler et al. immunoassay was unable to detect *Staphylococcus faecuim* at $5x10^6$ CFU/50µl ($1x10^8$ CFU/ml) suggesting that the antibodies are only able to cross-react with LTA on certain Staphylococcus *spp.* and Streptococcus *spp.*

In contrast, the following Tables set forth both the pangeneric cross-reactivity of the Verax antibodies, as well as the sensitivity of these antibodies, demonstrating efficacy at detecting clinically relevant amounts of bacteria in contaminated blood or blood products ($1x10^3$ CFU/ml – $1x10^5$ CFU/ml).

GRAM POSITIVE RAPID TEST SIGNAL (G/DENS)

CFU/ml	S. epidermidis	S. aureus	S. lugdenensis	B. cereus	GRP B Strep	GRP G Strep	S. pyogenes	E. faecalis	C. minutissimum	C. perfringes	CFU/ml
1.0 E5	17.26	1.04	13.21	12.24	0.26	16.07	nt	13.95	8.82	11.20	1.0 E5
5.0 E4	nt	nt	3.20	9.91	nt	nt	21.77	nt	nt	nt	5.0 E4
1.0 E4	8.81	0.41	0.38	2.45	0.04	1.43	10.32	3.19	2.03	8.67	1.0 E4
5.0 E3	3.98	nt	nt	nt	0.04	nt	nt	0.44	nt	nt	5.0 E3
1.0 E3	0.83	0.24	0.17	0.29	nt	0.46	0.51	0.27	1.26	2.91	1.0 E3

*BOXED CELL = MINIMALL DETECTABLE CONCENTRATION
**G/DENS = REFLECTANCE SIGNAL, ANY G/DENS > 0.25 IS VISIBLE
***nt = NOT TESTED

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII

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of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Jeffrey A. Hall

Dated: Signature:

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